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CLAIMS:

1. A process for preparing a cell capable of stable high yield expression of a target gene product having an essentially human glycosylation pattern, which method comprises:
(a) selecting an immortalized human cell or human hybrid cell (starting cell) which is derived from B lymphocytes and is capable of stable high yield expression of an immunoglobulin (Ig) being non-essential to the starting cell;
(b) screening for the locus of the Ig gene within the genome of the starting cell;
(c1) replacing the gene coding for the Ig with a first functional DNA sequence containing one or more recombinase recognition sites (RRS) to obtain a functionalized precursor cell; and
(d) integrating a second functional DNA sequence containing a DNA sequence coding for the target gene product into the functionalized precursor cell obtained in step (c1) by use of a recombinase recognizing the RRSs incorporated with the first functional sequence, or
(c2) directly replacing the gene coding for the Ig with a functional DNA sequence containing a DNA sequence coding for the target gene product.

2. The method of claim 1, wherein
(i) the starting cell secretes the Ig, preferably in an amount of at least 0.3 fmol/cell/d of a polypeptide chain and more preferably in an amount of more than 1 fmol/cell/d; and/or
(ii) if the starting cell is a human hybrid cell, the Ig gene is a human gene.

3. The method of claim 1 or 2 wherein the starting cell is a human myeloma or hybridoma, or human hetero-hybridoma cell and most preferably is human-mouse hetero-hybridoma H-CB-P1 (DSM ACC 2104).

4. The method according to any one of claims 1 to 3, wherein the integration of the functional DNA sequence(s) is effected at a rearranged Ig locus, preferably at a rearranged immunoglobulin H locus or λ locus of said starting cell.

5. The method according to any one of claims 1 to 4 wherein the locus of the Ig gene
(i) is known or is determined by a screening procedure comprising microarray expression analysis, 2D protein gel electrophoresis, quantitative PCR, RNAse protection, northern blot, ELISA, western blot and combinations thereof; and/or
(ii) is selected as to provide for an essentially human glycosylation pattern.

6. The method according to any one of claims 1 to 5, wherein the replacement of the Ig gene is effected
(i) by an one step replacement strategy, wherein the starting cell is contacted with a vector construct containing the first functional sequence, said first functional sequence replacing the gene coding for the Ig; or
(ii) in a two- or multi-step strategy, wherein the gene coding for the Ig gene is deleted or inactivated and subsequently contacted with a vector construct containing the first functional sequence, said first functional sequence being incorporated at the site of the deleted/inactivated Ig.

7. The method of claim 1 or 6, wherein the first functional DNA sequence
(i) comprises one or more RRS(s) selected from loxP, frt, attL and attR sites of lambdoid phages, and recognition sites for resolvases or phage C31 integrase,preferably RRS(s) capable of unidirectional integration such as modified loxP sites, frt sites, etc.; and/or
(ii) further comprises functional sequences selected from marker sequences, secretion proteins, promoters, enhancers, splice signals, polyadenylation signals and IRES elements; and/or
(iii) is flanked in the vector by sequences that are homologous to the target gene or adjacent sequences.

8. The method of any one of claims 1 to 7, wherein
(i) the integration of the second functional DNA sequence is effected by delivering a recombinases recognising the RRS(s) present in the first functional sequence together with, shortly before or after delivery of the second functional sequence;
(ii) the integrase is selected from Cre, Flp, ϕC31 integrase, resolvase, etc.;

(iii) the target gene product is selected from enzymes, hormones, cytokines, receptors, antibodies, antibody domains, fusion proteins comprising the gene product mentioned before, etc.
(iv) the second functional DNA sequence further comprises functional sequences selected from promoter sequences, marker sequences, splice donor and acceptor sequences, recombinase recognition sequences differing from RRS of the first functional sequence, etc.

9. The method of any one of claims 1 to 6, wherein the gene coding for the Ig is directly replaced with a functional DNA sequence containing a DNA sequence coding for the target gene product.

10. A method for preparing a functionalized cell comprising the steps (a) to (c1) as defined in claims 1 to 6.

11. A functionalized cell as defined in claim 10.

12. A cell capable of high yield expression of a target gene product obtainable by the method of claims 1 to 9.

13. The cell of claim 12, wherein the target gene product is an antibody, preferably the cell is PBG04 (DMS ACC2577).

14. The cell of claims 11, 12 or 13, which is derived from H-CB-P1 (DSM ACC2104).

15. The cell of claim 13 or 14 further having its light chain inactivated or replaced with a gene coding for the same or a different target gene product.

16. A method for high yield expression of a target gene product which comprises cultivating a cell as defined in any one of claims 12 to 15.

17. A target gene product obtainable by cultivating a cell as defined in claim 14 or 15.